SPERMATOGONIAL TRANSPLANTATION AS A NOVEL TECHNIQUE IN AQUACULTURE AND FISH CONSERVATION

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TRANSPLANTACIJA SPERMATOGONIJA KAO NOVA METODA U AKVAKULTURI I KONZERVACIJI RIBA

Apstrakt

Poslednjih godina, primena primordijalnih germinativnih ćelija (primordial germ cells - PGCs) i spermatogonijalnih stem ćelija (spermatogonial stem cells - SSCs) riba je postala veoma značajna zbog razvoja metode transplantacije ovih ćelija. Od kada su Brinster i Avarbock (1994) razvili ovu metodu, ona se uspešno koristi za čuvanje genetskog materijala ugroženih vrsta i u stvaranju novih transgenih linija kod miševa i domaćih životinja. Uvođenje ove metode kod riba predstavlja značajan napredak u oblasti reproduktivne biotehnologije, akvakulture, konzervacione biologije, kao i u razvoju novih transgenih linija različitih vrsta riba.

Osnovu ove metode predstavlja transplantacija germinativnih ćelija (PGC, SSC) iz donorskog organizma u organizam primaoca. Najinteresantnija u tom smislu je upotreba nediferenciranih spermatogonija A tipa (A_{und}) koje imaju sposobnost samoobnavljanja ali i proizvodnje ćelija kasnijih faza spermatogeneze. Postoji takođe nekoliko specifičnih osobina SSC koje ih čine pogodnim za transplantaciju: (1) sposobnost da kolonizuju testis primaoca odakle produkuju donorsku spermu, (2) mogućnost da se nakon transplantacije u primaocu muškog pola razviju u spermatogonije, a u primaocu ženskog pola u oogonije i (3) mogućnost genske manipulacije sa ciljem produkcije transgenih riba (Lacerda i sar., 2010).

Prilikom transplantacije SSC, posebna pažnja mora biti usmerena ka izboru vrste donora i primaoca. Najbolje bi bilo da donor i primalac ne budu filogenetski previše udaljeni, kao i da primalac ima kratak reproduktivni ciklus i manje dimenzije tela kako bi ekonomski bio pogodniji za gajenje. Donorska vrsta je obično vrsta za koju postoji određneni interes, bilo ekonomski, naučni ili konzervacioni.

Tokom transplantacije, kompatibilnost između primaoca i donora može biti ograničavajući faktor u uspehu samog procesa. U najgorem slučaju, primalac, usled imune reakcije, može u potpunosti odbaciti transplantirano tkivo ili ćelije. To je najčešće slučaj ukoliko se vrši transplantacija SSC iz odraslog donora u odraslog primaoca.

Kako bi se izbegao problem izazvan transplantacijom između dve odrasle jedinke, koristi se prednost ontogenije primaoca, posebno ontogenije njegovog imunog sistema, tako što se za primaoca koriste embrioni ili larve. Ovi stadijumi kod riba nemaju razvijen imuni sistem niti diferencirane T-ćelije (Takeuchi et al., 2003; Yoshizaki et al., 2011) te s toga nemaju mehanizam pomoću kojeg bi odbacili donorsko tkivo. Takođe, lakše je blokirati razvoj endogenih primordijalnih germinativnih ćelija kod larvi, nego ukloniti SSC iz već razvijenih gonada kod odraslog donora.

Pored odabira odgovarajuće vrste donora i primaoca, neophodno je na pravi način izolovati specifične ćelije koje treba da budu transplantirane. Ovaj proces je donekle jednostavniji kada je u pitanju tansplantacija PGC s obzirom na njihov daleko manji broj u odnosu na spermatogonije i na to da one još uvek nisu potpuno razvijene u gonadama. S druge strane, SSC su dobro razvijene u gonadama i najčešće zauzimaju karakteristično mesto unutar pojedinačnih niša u testisu specifičnih za tu vrstu ćelija. Prilikom izolacije nediferenciranih spermatogonija A tipa iz testisa odrasle jedinke, veome je bitno voditi računa od morfologiji tih ćelija kao i specifičnim markerima pomoću kojih ih je moguće razlikovati od ostalih tipova spermatogonija (A_{diff}, B), spermatocita i spermatida. Osnovne histološke metode u kombinaciji sa imunohistohemijom, *in situ* hibridizacijom ili *in situ* PCR metodom se mogu koristiti za identifikaciju spefifičnih molekularnih markera (proteina ili RNK) u ćelijama unutar ćelijskih niša i koji se u daljem toku rada mogu koristiti za izolaciju određenih ćelija.

Pre transplantacije PGC ili SSC, neophodno je izolovati željene ćelije iz donorskog tkiva. Nakon multienzimske razgradnje tkiva testisa, ćelije se izoluju na osnovu njihove morfologije i veličine i/ili specifičnih molekularnih markera zbog kojih čitav proces može biti species-specifičan.

Kombinacija transplantacije PGS i SSC sa krioprezervacijom daje dodatni značaj ovoj metodi s obzirom da još uvek ne postoji optimizovan protokol za uspešnu krioprezervaciju jaja i embriona riba, pre svega zbog prisustva velike količine žumanceta i masti.

Krioprezervacija ćelija kao što su PGS i SSC, koje imaju mogućnost da produkuju spermatozoide ili oocite u zavisnosti od pola jedinke primaoca, ima izuzetno veliku perspektivu primene u konzervacionoj biologiji i akvakulturi. Istraživanja su pokazala da krioprezervirane SSC nakon odmrzavanja i transplantacije u telo primaoca mogu proizvesti spermatozoide i oocite donorske vrste (Kobayashi et al., 2007). Na taj način, čuvanje gameta nije neophodno jer krioprezervacijom germinativnih ćelija i njihovom transplantacijom, moguće je dobiti gamete oba pola.

Ključne reči: spermatogonija, izolacija, primalac, donor

Abstract

In recent years, the importance of manipulations of primordial germ cells (PGCs) and spermatogonial stem cells (SSCs) in fish has drastically increased due to development of transplantation method of these cells. Since its development by Brinster and Avarbock (1994), this method has been successfully used in the preservation of genetic material of

endangered species and in the creation of new transgenic lines of mice and farm animals. Introduction of this method in fish leads to advances in reproductive biotechnology, aquaculture, development of new transgenic lines and conservation biology of fish.

The base of this method lies in the transplantation of the germinative cells (PGCs, SSCs) from donor organism into recipient organism. Undifferentiated spermatogonia type A (A_{und}) which have the ability of self-renewal are the most interesting for transplantation since they have the ability of self-renewal, but can also produce later stage cells.

There are several advantages of using SSCs in transplantation process: (1) the capability of SSCs to colonize the testis of the recipients where they are able to produce donor-derived sperm, (2) plasticity in development since SSCs can develop into spermatogonia in male recipients and oogonia in female recipients and (3) the possibility of genetic manipulation in SSCs in order to produce transgenic fish (Lacerda et al., 2010).

When transplanting SSCs, special attention must be given to the choice of donors and recipients species. It is best that donor and recipient organisms are phylogenetically not too distant, that recipient organisms have a short reproductive cycle and that they are small for a more economic rearing. Donor species are usually species which attract certain interest, whether its an economic, scientific or conservation interest.

During transplantation, compatibility between recipient and donor may be a very limiting factor in transplantation success. In the worst-case scenario, recipients may completely reject the transplanted tissue or cells due to immunological reaction. This is especially the case when transplanting SSCs isolated from adult donors into adult recipients. In order to evade the problems caused by adult-adult transplantations, scientists have taken advantage of the ontogeny of recipients, mainly the ontogeny of their immune system, and used embryos and larvae as recipients. Embryos and larvae do not have a developed immune system nor differentiated T-cells (Takeuchi et al., 2003; Yoshizaki et al., 2011), therefore they do not have mechanisms to reject the donor tissue. Furthermore, it is easier to knock-out larval endogenous PGCs than to deplete SSCs from already developed gonad.

Apart from choosing the right donor and recipient organisms, it is necessary to isolate specific cells that need to be transplanted. This is to some extent easier when transplanting PGCs, since there are fewer of them than SSCs, and they have not yet fully developed inside the gonads. On the other hand, SSCs are well developed inside the gonads and usually take their specific place within the spermatogonial stem niche. When isolating undifferentiated spermatogonia type A from adult testis, special attention must be given to their morphology and specific markers that distinguish them from other types of spermatogonia (A_{diff}, B), spermatocytes and spermatids. Basic histology may be coupled with immunohistochemistry, in situ hybridization or in situ PCR which would enable the identification of specific molecular markers within the cells of the niche (proteins or RNA). All this data can be further used in isolation of particular cells.

Prior to transplantation, PGCs and spermatogonia need to be isolated from the donor tissue. After multi-enzymatic digestion it is possible to isolate cells based on their morphology and size, and/or specific molecular markers and the whole process can be species-specific.

A great advantage of transplantation of PGCs and SSCs is that this method can be very well combined with cryopreservation. There are still no optimized protocols for cryopreservation of fish eggs and embryos, mostly due to presence of large amount of yolk and fat. Since PGCs and SSCs can develop into both sperm and eggs, cryopreservation of these

cells could have a great perspective in conservation biology but also in aquaculture. Studies have shown that frozen/thawed SSCs transplanted into recipients give rise to potent donor sperm and eggs in the recipients (Kobayashi et al., 2007). In this way, there is no need to conserve both sperm and eggs since successful cryopreservation of germ cells can give rise to both sperm and eggs after transplantation.

Keywords: spermatogonia, isolation, recipients, donor

ACKNOWLEDGEMENTS

The work was supported by the COST Action FA1205 AQUAGAMETE and the project 8526-5/2014/TUDPOL of the Ministry of Human Resources of Hungary awarded to Szent István University.

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